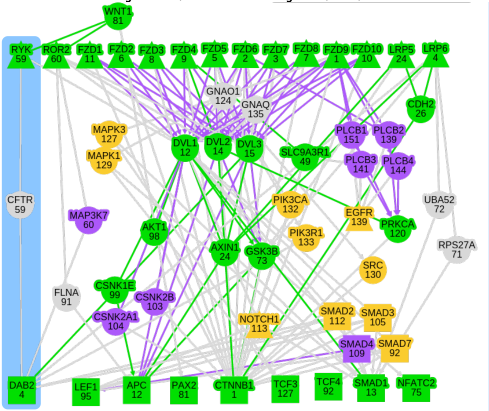
# CS 5854: PathLinker

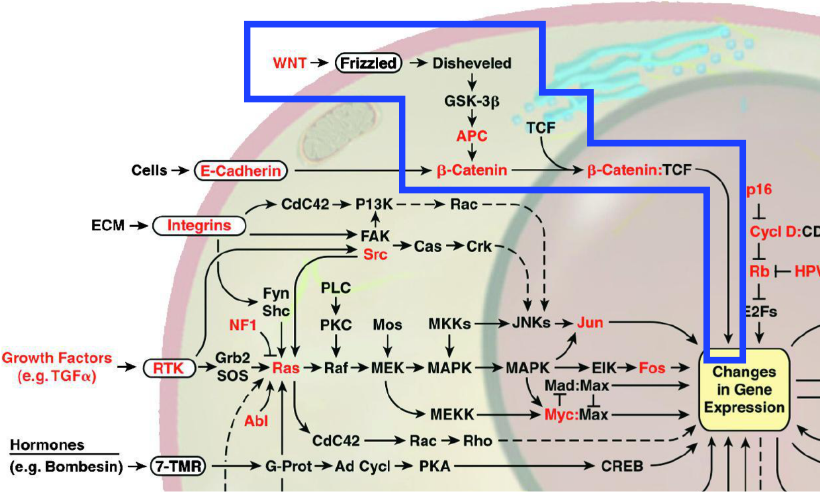
## Automated Reconstruction of Human Signaling Networks

T. M. Murali

January 30, February 1, 6, 2023



# Wnt Pathway



*Hanahan and Wienberg. Hallmarks of cancer. Cell, 2000.*

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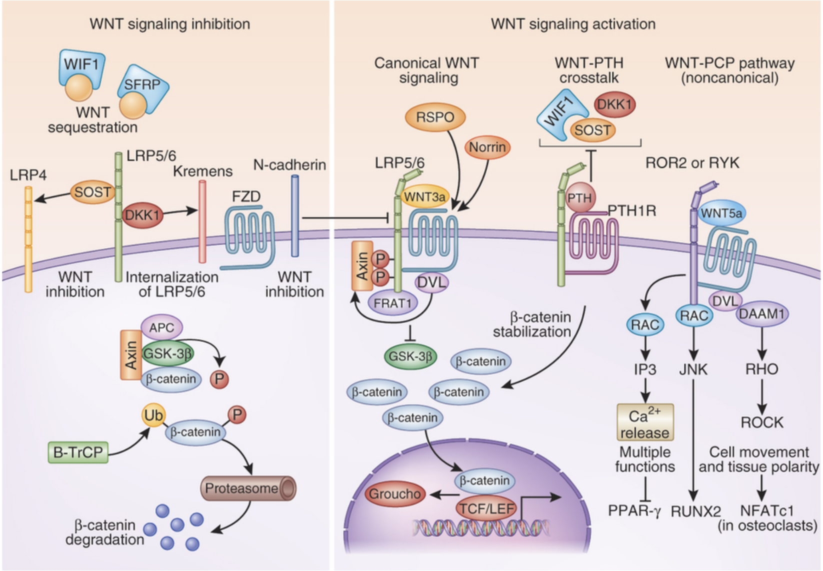
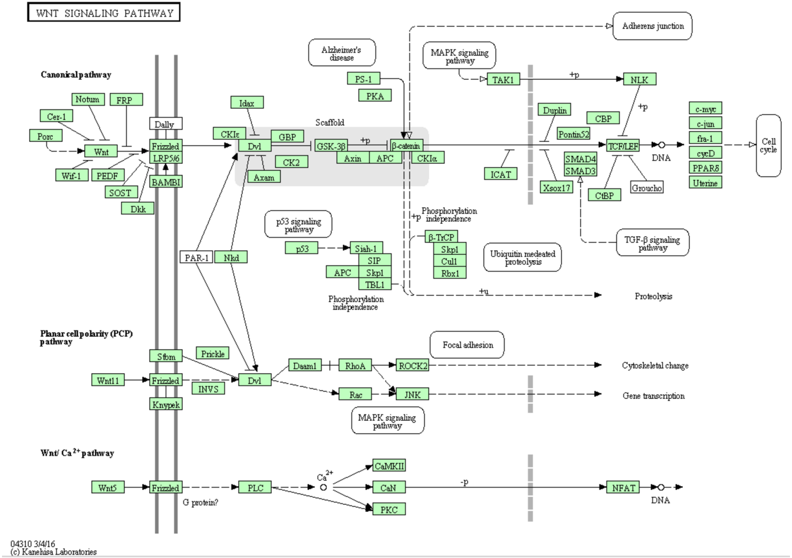


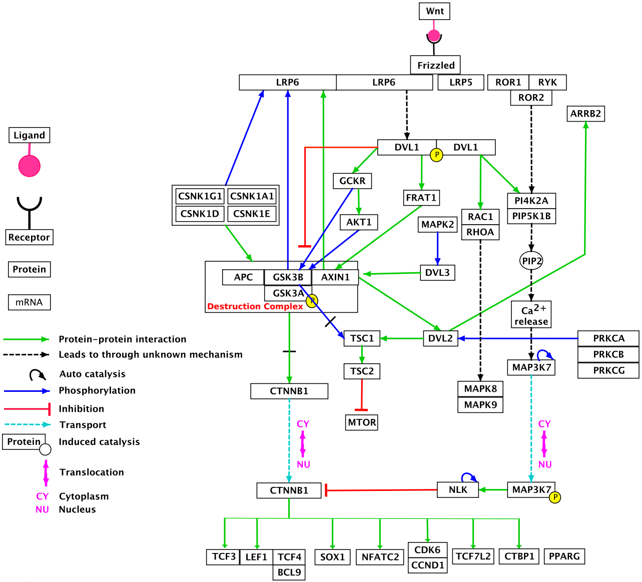
*Baron and Kneissel. WNT signaling in bone homeostasis and disease: from human mutations to treatments. Nat. Med., 2013.*

# Wnt Signaling in a Pathway Database



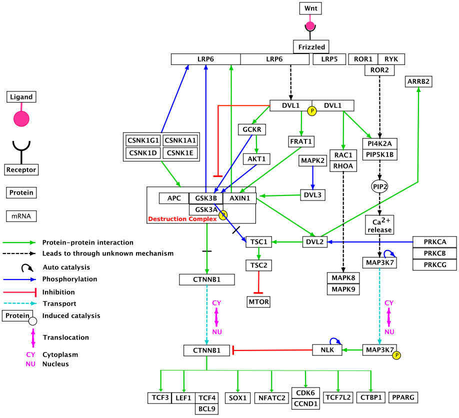
*KEGG database*

# Wnt Signaling in a Pathway Database

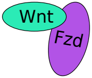


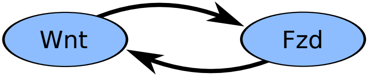
*www.netpath.org/netslim*

# Signaling Pathways as Directed Graphs

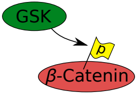


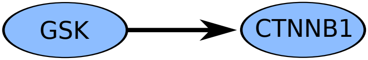
## Bidirected Physical Interactions



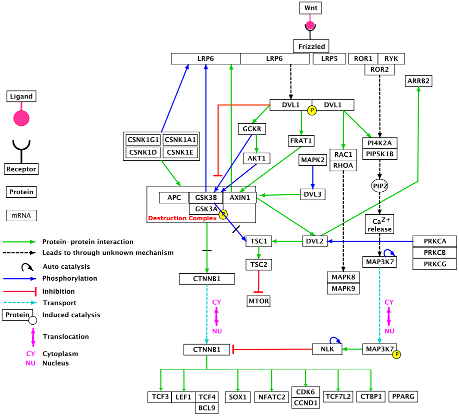


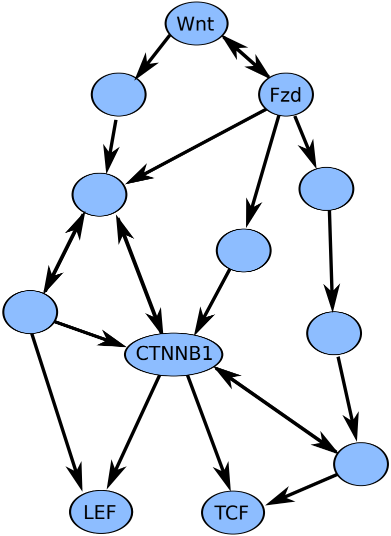
## Directed Regulatory Interactions





# Signaling Pathways as Directed Graphs





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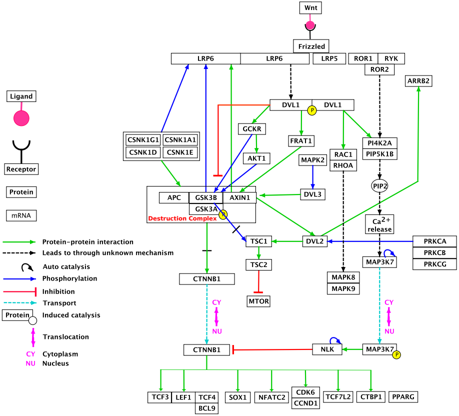
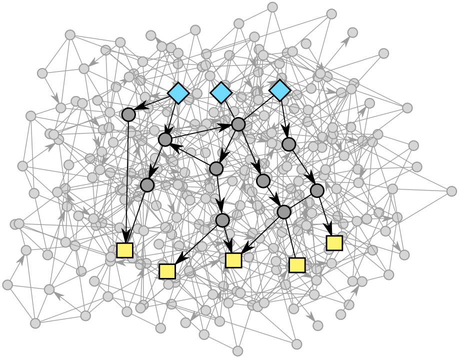
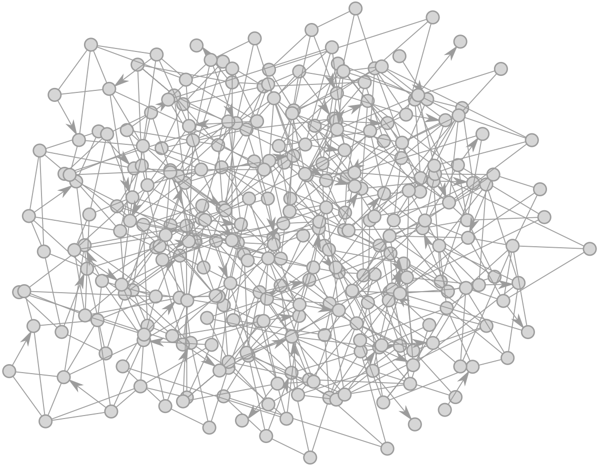


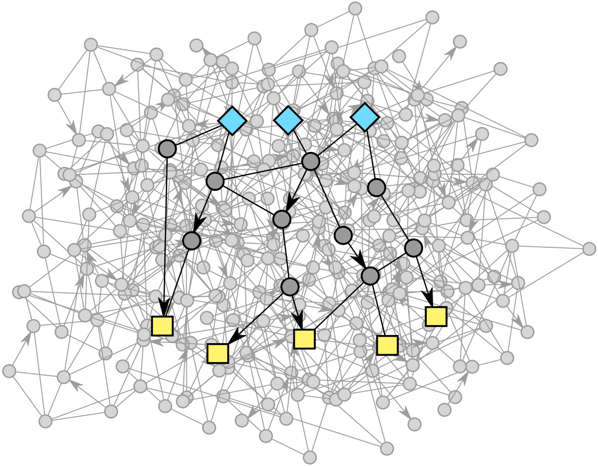


# Reconstructing Signaling Pathways



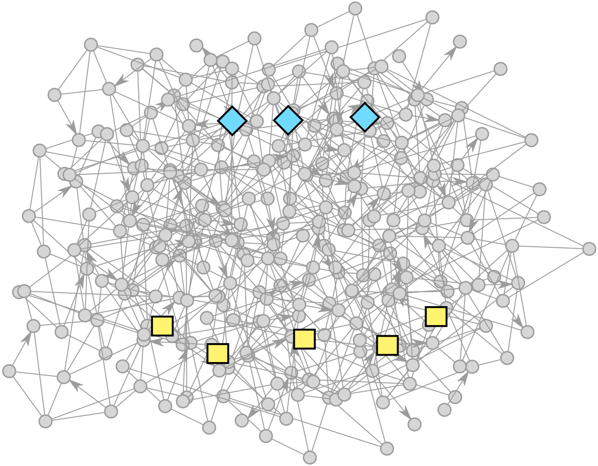
Human protein-protein interaction network
All known interactions among human proteins

# Reconstructing Signaling Pathways



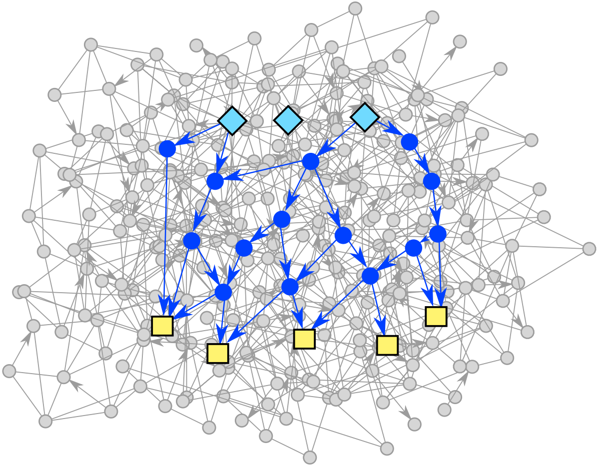
A pathway is a subgraph of the interaction network

# Reconstructing Signaling Pathways



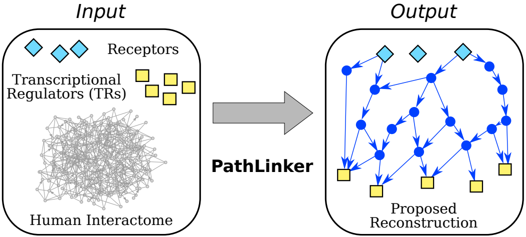
**Question:** Can we reconstruct the pathway given only receptors and transcriptional factors?

# Reconstructing Signaling Pathways



Proposed pathway reconstruction

# Automated Reconstruction of Signaling Pathways



- Developed **PathLinker** to reconstruct *proteins and interactions*
- **Systematically evaluated** PATHLINKER and other algorithms on human signaling pathways from the NetPath and KEGG databases

*"Pathways on Demand: Automatic Reconstruction of Human Signaling Pathways," Ritz et al., Systems Biology and Applications, a Nature partner journal, 2, 16002, 2016.*

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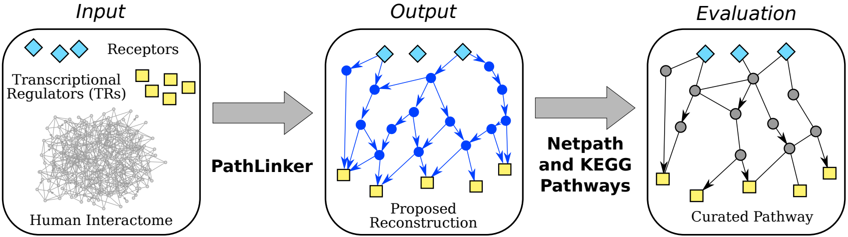

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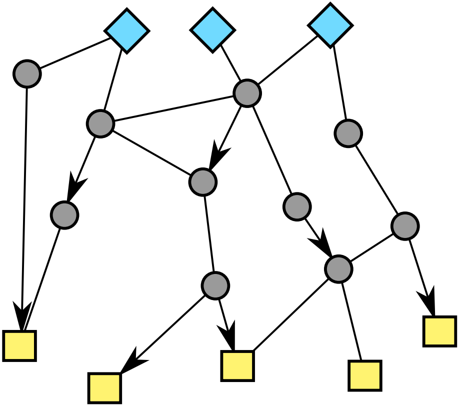
# Evaluation of Reconstructed Pathways



Curated Pathway

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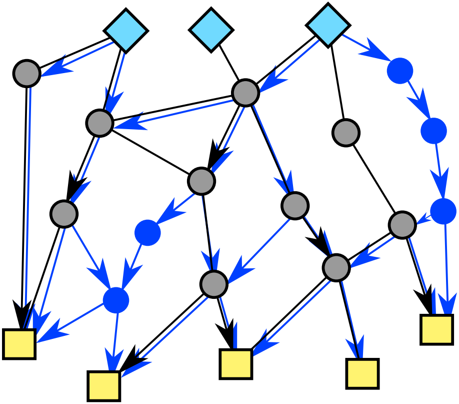

Curated Pathway and Proposed Reconstruction

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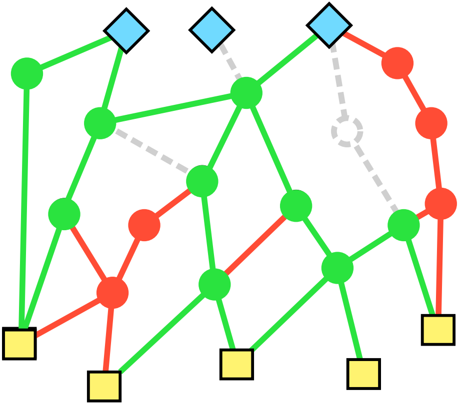

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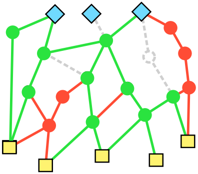

Recall:

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Precision:

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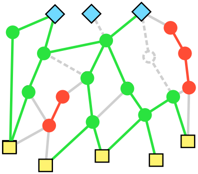

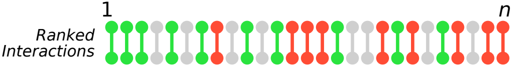

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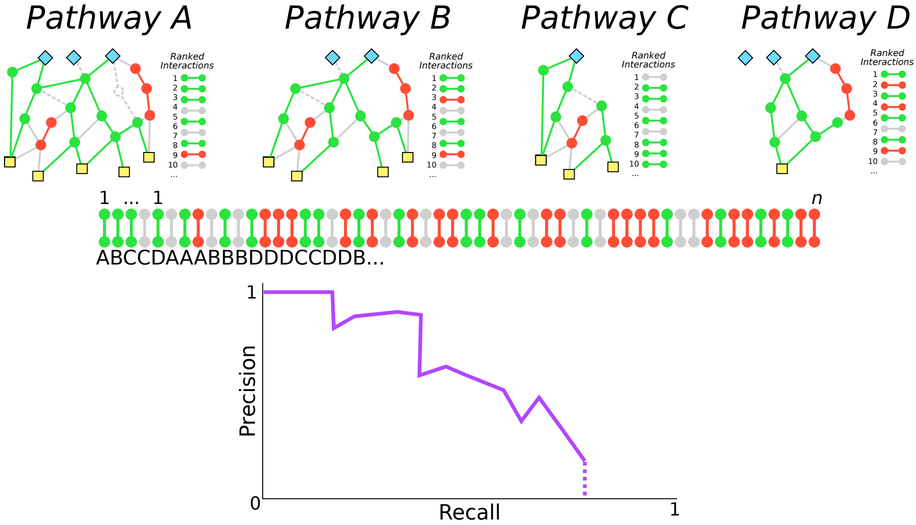
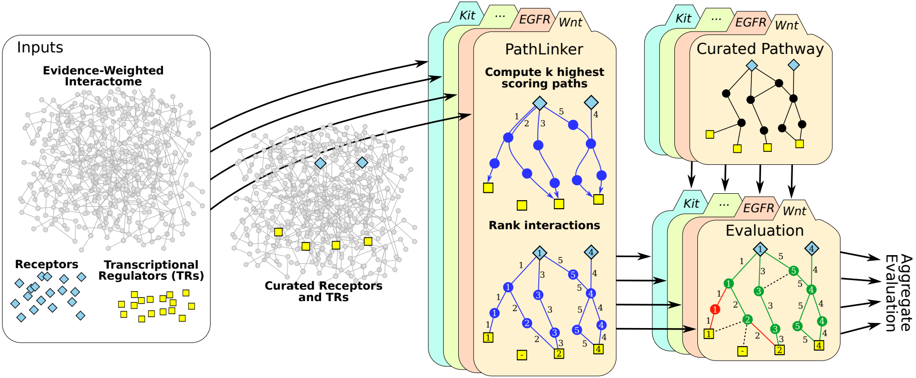
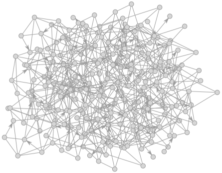
# Evaluating Multiple Reconstructions



# Complete Pipeline



# Inputs for Pathway Reconstruction



Protein-Protein Interactome

- 12K nodes and 152K directed edges
- 61K physical interactions[1–4]
  BIND, DIP, InnateDB, IntAct, MINT, MatrixDB, Reactome, NetPath, KEGG, SPIKE
- 30K signaling interactions[2–4]
  NetPath, KEGG, SPIKE

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[1] *Aranda et al., PSICQUIC and PSISSCORE: assessing and scoring molecular interactions. Nature Methods, 2011.*
[2] *Kandasmy et al., NetPath: a public resource of curated signaling transduction pathways. Genome Biology, 2010.*
[3] *Kanehisa et al., KEGG for integration and interpretation of large-scale molecular data sets. Nucleic Acids Research, 2012.*
[4] *Paz et al., SPIKE: a database of highly curated human signaling pathways. Nucleic Acids Research, 2009.*

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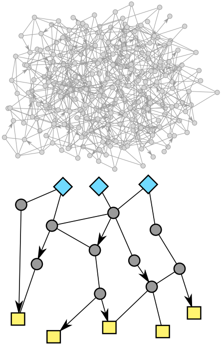

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Signaling Pathways from NetPath[2]

- 15 immune and cancer pathways

▸ List of NetPath Pathways

2,124 Receptors[5]

2,286 Transcriptional Regulators[6,7]

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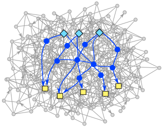
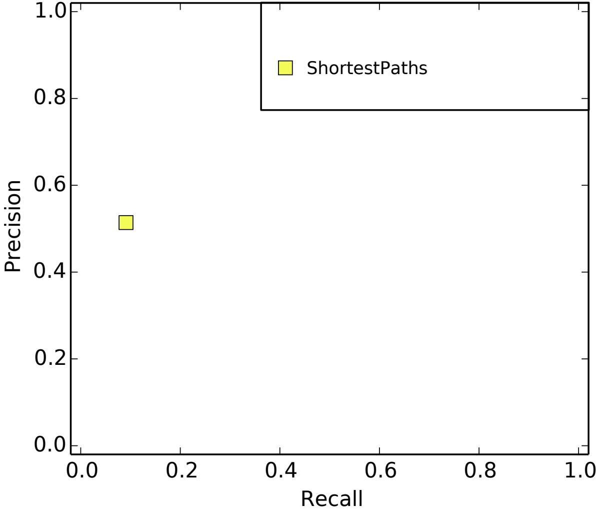
[1] *Aranda et al., PSICQUIC and PSISSCORE: assessing and scoring molecular interactions. Nature Methods, 2011.*
[2] *Kandasmy et al., NetPath: a public resource of curated signaling transduction pathways. Genome Biology, 2010.*
[3] *Kanehisa et al., KEGG for integration and interpretation of large-scale molecular data sets. Nucleic Acids Research, 2012.*
[4] *Paz et al., SPIKE: a database of highly curated human signaling pathways. Nucleic Acids Research, 2009.*
[5] *Almen et al., Mapping the human membrane proteome: a majority of the human membrane proteins can be classified according to function and evolutionary origin. BMC Biology, 2009.*
[6] *Ravasi et al., An atlas of combinatorial transcriptional regulation in mouse and man. Cell, 2010.*
[7] *Vaquerizas et al., A census of human transcription factors; function, expression and evolution. Nature Review Genetics, 2009.*

# Results

ShortestPaths

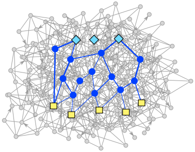


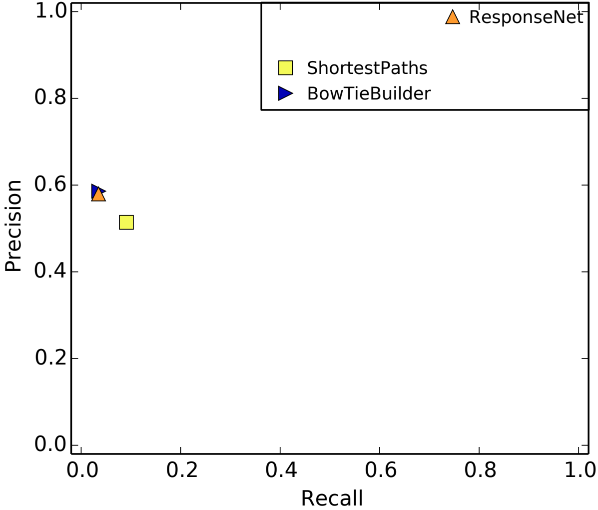


*Steffen et al., Automated modelling of signal transduction networks. BMC Bioinformatics, 2002.*

# Results

Network Flow:
RESPONSENET

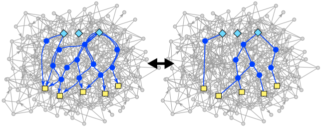




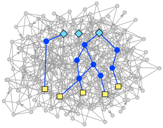
*Yeger-Lotem et al., Bridging high-throughput genetic and transcriptional data reveals cellular responses to alpha-synuclein toxicity. Nature Genetics, 2009.*

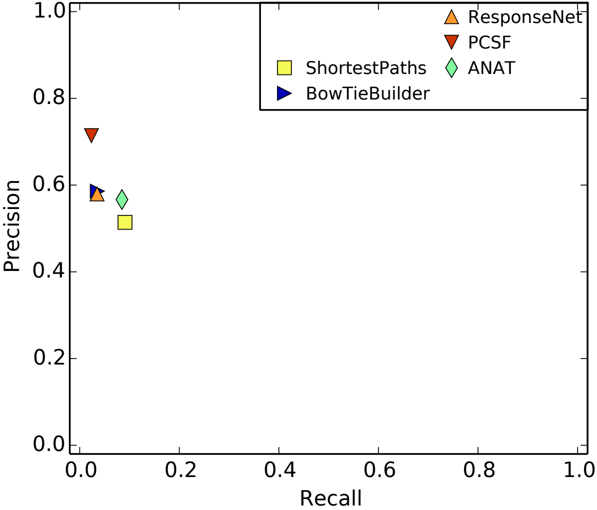
# Results

Between SHORTESTPATHS and Steiner Tree: ANAT



Prize Collecting Steiner Forest (PCSF)

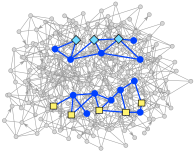


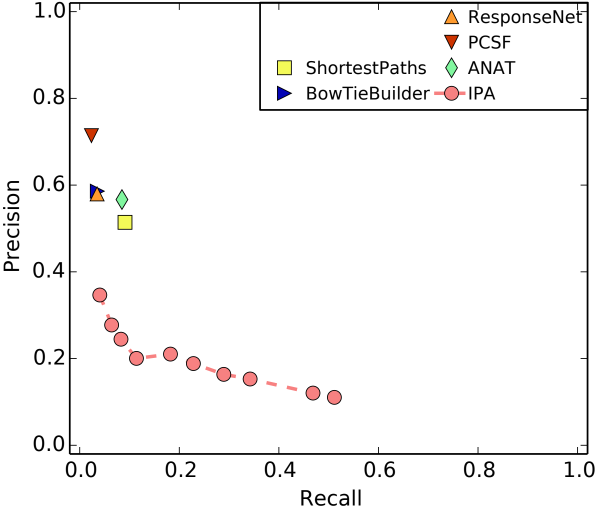


*Yosef et al., ANAT: A tool for constructing and analyzing functional protein networks. Science Signaling, 2011.*
*Tuncbag et al., Simultaneous reconstruction of multiple signaling pathways via the prize-collecting Steiner forest problem. Journal of Computational Biology, 2013.*

# Results

Ingenuity Pathway Analyzer (IPA)

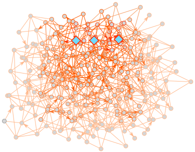


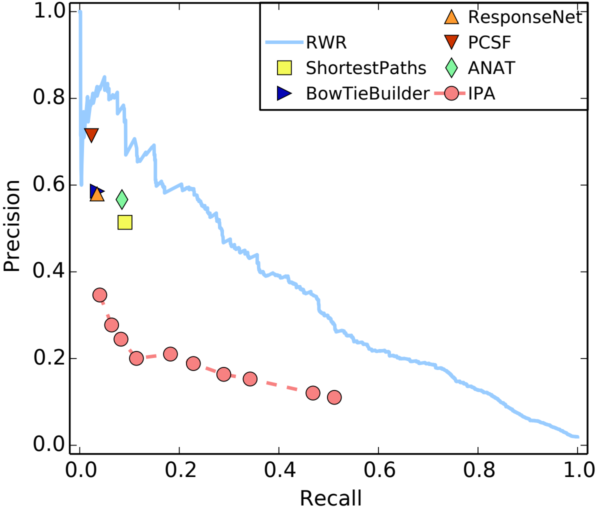


*Ingenuity Pathway Analysis. IPA Network Generation Algorithm. White Paper, 2005.*

# Results

Random Walk with Restarts (RWR)

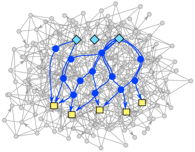


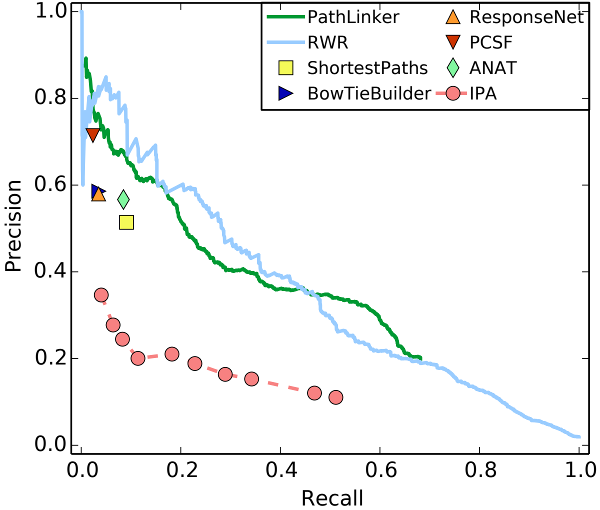


*Page et al., The PageRank citation ranking: Bringing order to the web. Technical Report, 1999.*

# Results

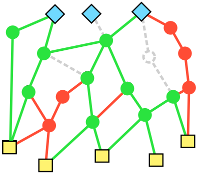
**PathLinker**





*Yen. Finding the k shortest loopless paths in a network. Management Science, 1971.*
**This paper.**

# Evaluation of Reconstructed Pathways



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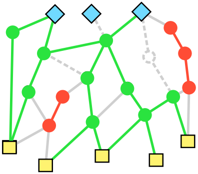

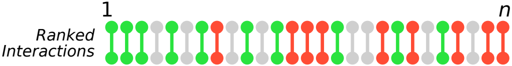

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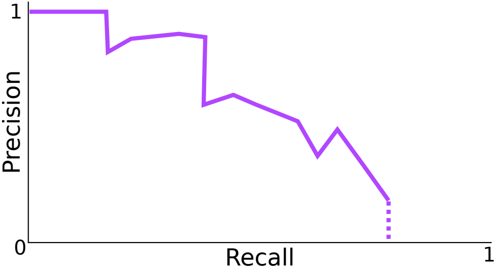

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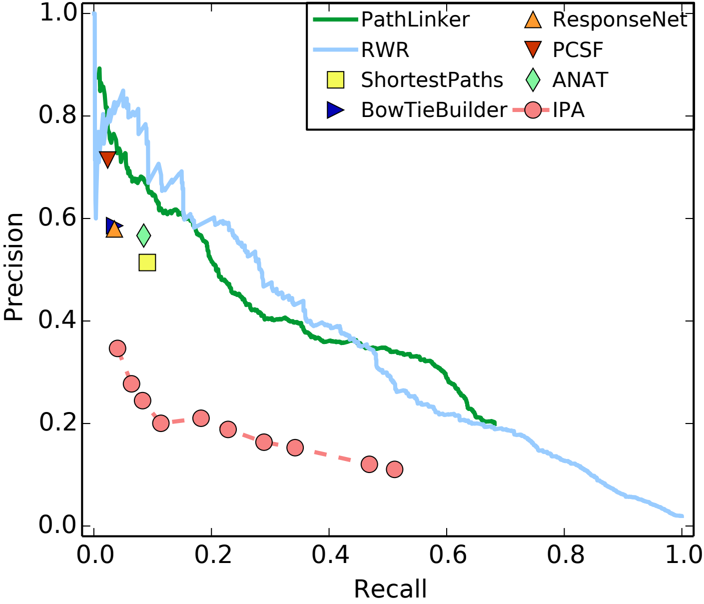
# Ignore Pathway-Adjacent Negatives



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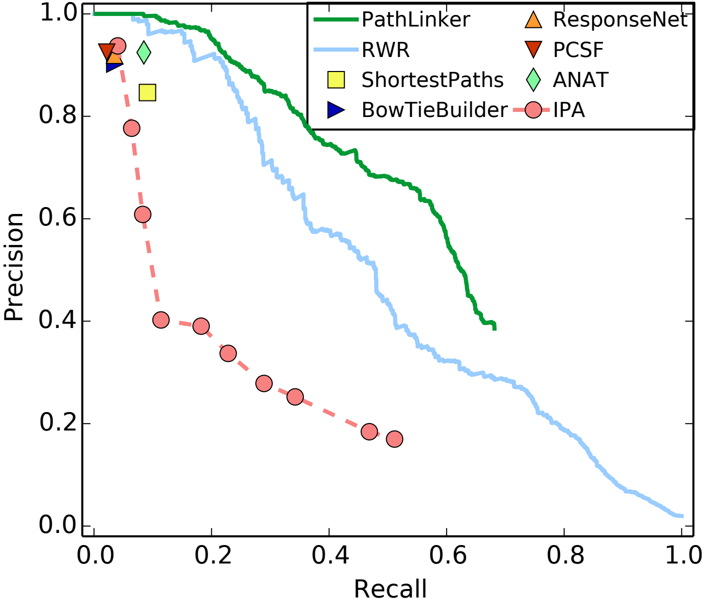
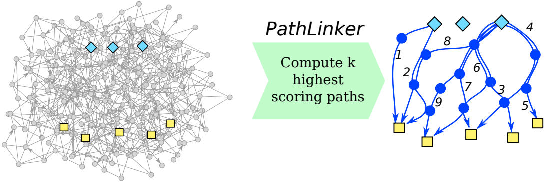


# Why does PathLinker improve over other methods?


*PathLinker*

Compute k highest scoring paths

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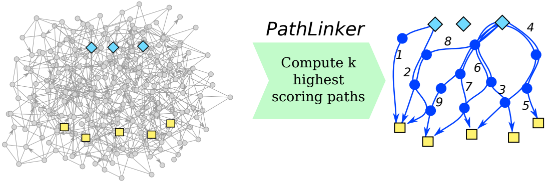

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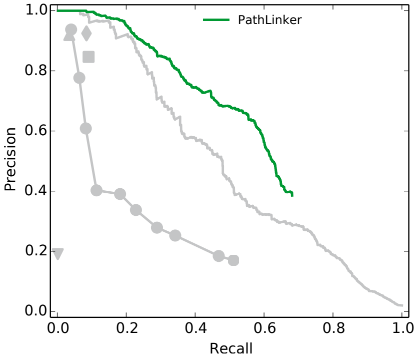

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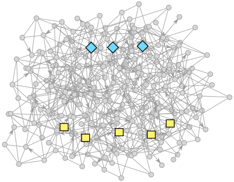
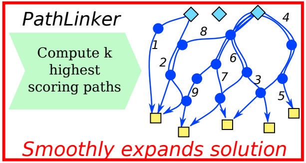
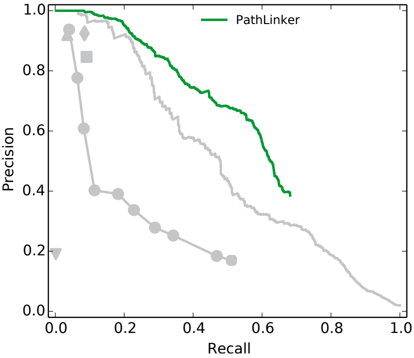




Compute k highest scoring paths

*Smoothly expands solution*



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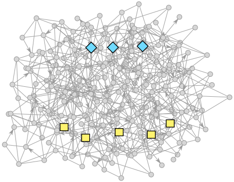

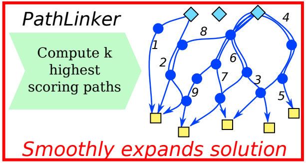
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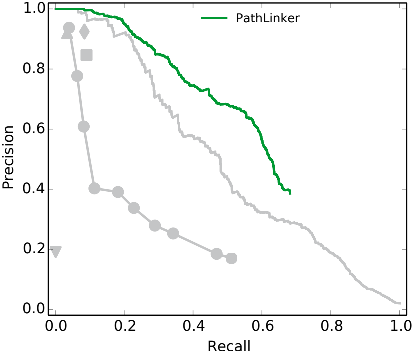

▸ Parameter sweep

# Algorithms Compared

| Abbreviation | Algorithm Type |
|---|---|
| ShortestPaths | Shortest paths from every receptor to every TR |
| PathLinker | *k* shortest paths from any receptor to any TR |
| RWR | Random walk with restarts (aka PageRank) |
| ResponseNet | Network flow |
| ANAT | Tradeoff between shortest paths and Steiner trees |
| PCSF | Prize-Collecting Steiner Forest |
| IPA | Ingenuity Pathway Analyzer: grow subnetworks greedily |
| BowTieBuilder | Approximation to the Steiner tree connecting receptors and TRs |

# Algorithms Compared

| **Abbreviation** | **Algorithm Type** |
|---|---|
| ShortestPaths | Shortest paths from every receptor to every TR |
| PathLinker | $k$ shortest paths from any receptor to any TR |
| RWR | Random walk with restarts (aka PageRank) |
| ResponseNet | Network flow |
| ANAT | Tradeoff between shortest paths and Steiner trees |
| PCSF | Prize-Collecting Steiner Forest |
| IPA | Ingenuity Pathway Analyzer: grow subnetworks greedily |
| BowTieBuilder | Approximation to the Steiner tree connecting receptors and TRs |

# Drunkard's Walk

- A drunk person leaves a bar.
- They move in steps, either by one unit to the right or by one unit to the left.
- When will they reach their home at the end of the street?
- If they return to the bar, they can only step out.

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- If they return to the bar, they can only step out.
- How do we think about this problem?
- Street is the $x$-axis, bar is at $x = 0$, house is at $x = n$.
- Where could the drunk be after 1 step? After 2 steps? After 3 steps? After $k$ steps?
- What is the probability that the drunk reaches home after $k$ steps?
- What is the probability that the drunk reaches home at all?

# Random Walk on a Grid

- A random walker leaves a starting location (conveniently at $(0, 0)$).
- They move in steps, either by one unit to the right, left, top, or bottom.
- When will they reach their destination, which is at $(n, n)$?

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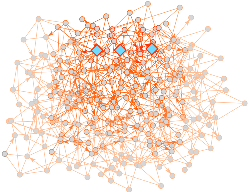
- Where could the walker be after 1 step? After 2 steps? After 3 steps? After $k$ steps?
- What is the probability that the walker reaches their destination after $k$ steps?
- Convenient to think of the grid as a graph. Can generalise the problem to a graph.

# RWR Algorithm

Given weighted, directed graph $G = (V, E, W)$, receptors $S \subset V$ and TRs $T \subset V$, and a parameter $0 \leq q < 1$.

- Walker at $u$ transitions as follows:

Walk: With prob. $1 - q$, walk to neighbor $x$ with prob. $w_{ux}/d_u$ (outdegree)



*Page et al., The PageRank citation ranking: Bringing order to the web. Technical Report, 1999.*

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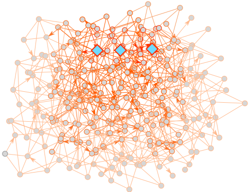

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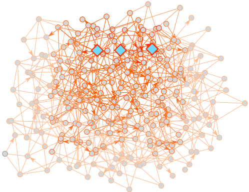

- Compute the probability $p(v)$ each node is visited as steps $\rightarrow \infty$.

$$p(v) = \frac{q}{|S|}[v \in S] + (1 - q) \sum_{u \in N_v^{\text{in}}} \frac{w_{uv}}{d_u} p(u).$$

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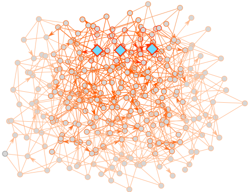

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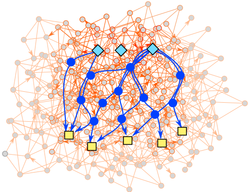
- Output edges in decreasing order of edge fluxes: $f_{uv} = p_u w_{uv}$

*Page et al., The PageRank citation ranking: Bringing order to the web. Technical Report, 1999.*

# PathLinker Algorithm

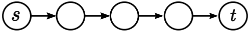
Given weighted, directed graph $G = (V, E, W)$, receptors $S \subset V$ and TRs $T \subset V$.

- Find the $k$ "highest-scoring" paths from any $s \in S$ to any $t \in T$.
- Replace Dijkstra's algorithm with the A* algorithm for significant practical speedup.



*Yen. Finding the k shortest loopless paths in a network. Management Science, 1971.*

# Shortest Loopless Paths – Basic Idea



- Naïve Approaches (time-consuming):
  - Enumerate all paths from $s$ to $t$ and sort.
  - Obtain $k - 1$ shortest paths, hide an edge from each path and find a shortest path in the modified network. Test all combinations.

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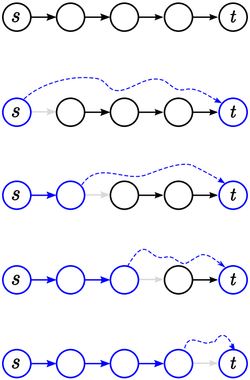

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  - Obtain $k-1$ shortest paths, hide an edge from each path and find a shortest path in the modified network. Test all combinations.
- Basic idea of Yen's algorithm:
  - Compute the shortest path from $s$ to $t$
  - The $k^{\mathrm{th}}$ shortest path will be a deviation from the previously-discovered shortest path.

# Shortest Loopless Paths

- $\{s, v_2, v_3, \ldots, t\}$ denotes a simple path from $s$ to $t$
- $P^k = \{s, P^k_2, P^k_3, \ldots, P^k_{|P^k|-1}, t\}$ is the $k^{\text{th}}$ shortest path from $s$ to $t$

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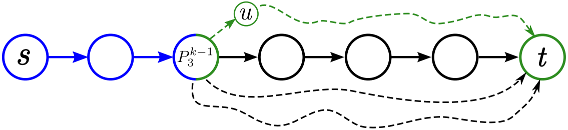
- $D^k_i$ is the "deviation from $P^{k-1}$ at node $P^{k-1}_i$"
  More specifically, the shortest $s \rightsquigarrow t$ path that:
  1. coincides with $P^{k-1}$ from $s$ to $P^{k-1}_i$
  2. deviates to a node $u$ where $u$ is not used as this deviation in any of the $k-1$ shortest paths
  3. reaches $t$ by a shortest path from $u$ without using any node in the first part of the path



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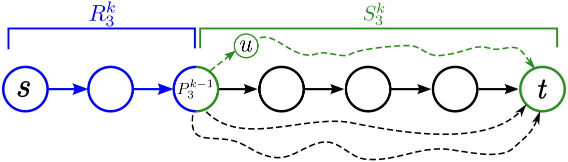
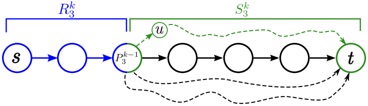


- $R^k_i = \{s, P^k_2, P^k_3, \ldots, P^k_i\}$ is the root of $D^k_i$
- $S^k_i = \{P^k_i, \ldots, t\}$ is the spur of $D^k_i$

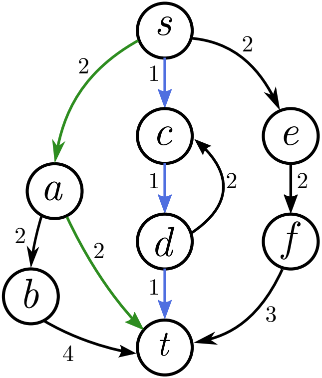
# Shortest Loopless Paths

- Find the shortest path $P^1$
- For $k = 2, 3, \ldots$, find $P^k$ as follows:

```
Let $B^k = B^{k-1}$, the set of candidate paths from iteration $k-1$
for $1 \leq i < |P^{k-1}|$ do
    Let $x = P_i^{k-1}$
    Hide incoming edges to $x$ for the remainder of iteration $k$
    for each $j$ such that the first $i$ nodes in in $P^j$ match $P^{k-1}$ do
        Hide edge $(x, P_{i+1}^j)$ for the remainder of iteration $k$
    end for
    $R_i^k$ is the first $i$ nodes of $P^{k-1}$
    $S_i^k$ is the shortest path from $x$ to $t$
    Join $R_i^k$ and $S_i^k$ to form $D_i^k$
    Add candidate path $D_i^k$ to $B^k$
end for
Remove the shortest path from $B^k$ and return it
```

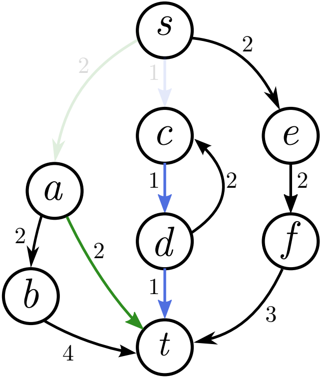


# Example – Find $P^3$



$$P^1 = \{s, c, d, t\}$$
$$P^2 = \{s, a, t\}$$
$$P^3 = \quad ?$$

# Example – Hide Edges for Root $\{s\}$



$P^1 = \{s, c, d, t\}$

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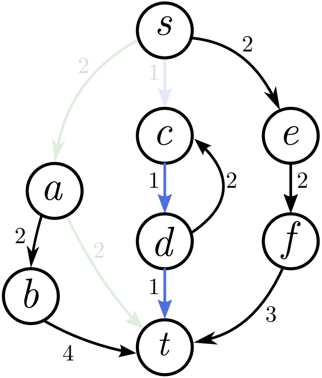
# Example – Hide Edges for Root $\{s, a\}$

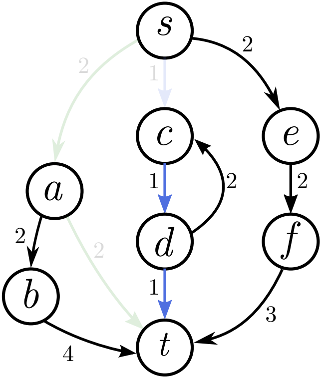


$P^1 = \{s, c, d, t\}$

$P^2 = \{s, a, t\}$

$P^3 =$ ?

# Example – Find Shortest Spur for Each Root



$P^1 = \{s, c, d, t\}$

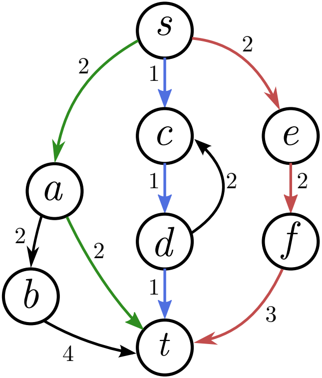
$P^2 = \{s, a, t\}$

$P^3 =$ ?

$S_1^3 = \{s, e, f, t\}$

$S_2^3 = \{a, b, t\}$

# Example – Identify Shortest Deviation



$P^1 = \{s, c, d, t\}$

$P^2 = \{s, a, t\}$

$P^3 = \quad ?$

$S_1^3 = \{s, e, f, t\}$

$S_2^3 = \{a, b, t\}$

$D_1^3 = \{s, e, f, t\}$

$D_2^3 = \{s, a, b, t\}$

# How do we find $S_i^k$ efficiently?

- For $k = 2, 3, \ldots$, find $P^k$ as follows:

```
Let $B^k = B^{k-1}$, the set of candidate paths from iteration $k - 1$
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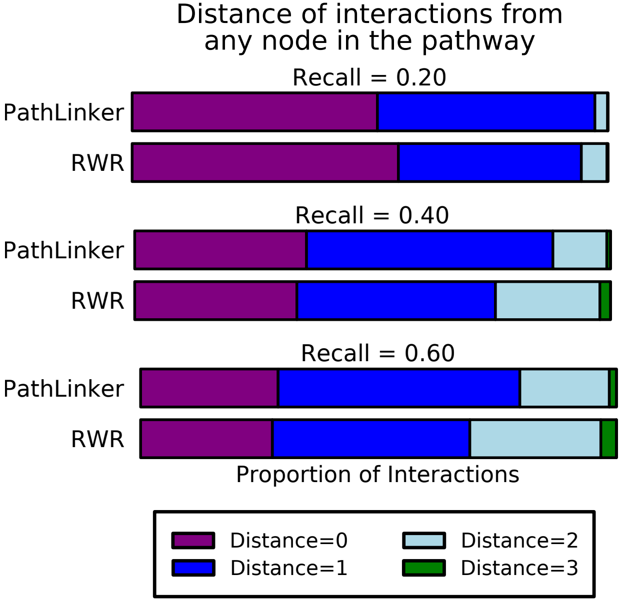
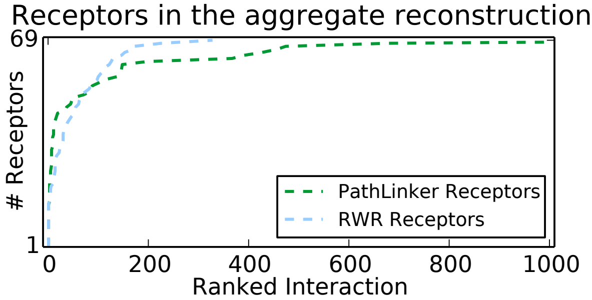
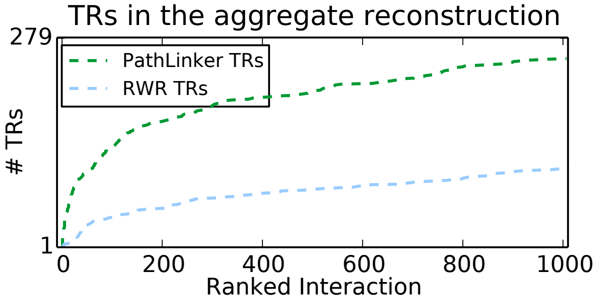
# Compare Distances to Curated Pathway

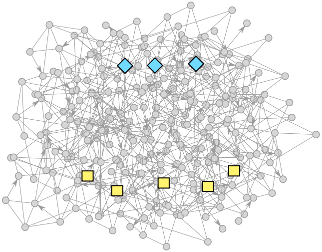


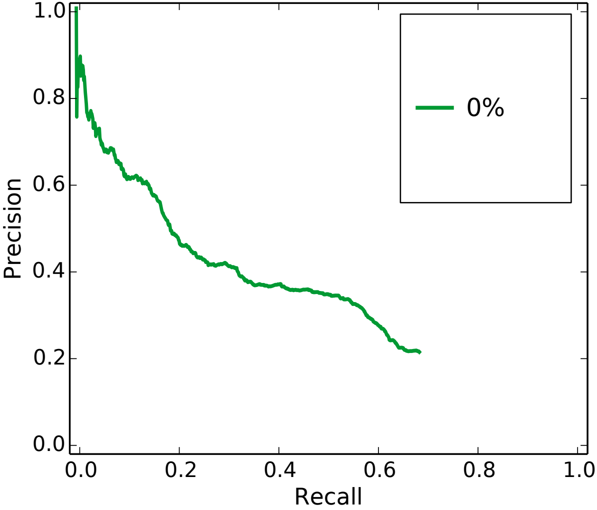
# Compare Rate of Recovery of Receptors/TRs





# Add Noise to Inputs





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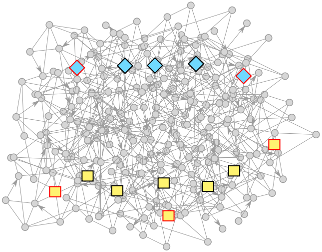

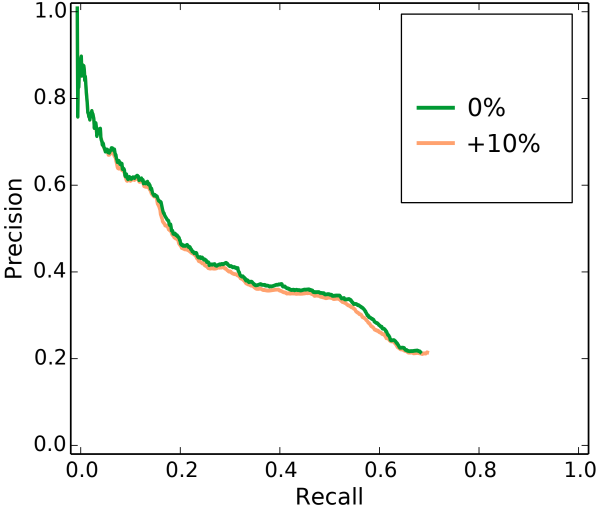

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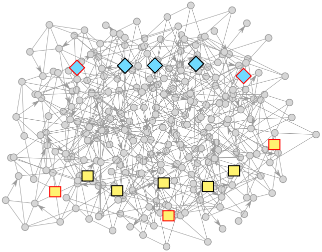

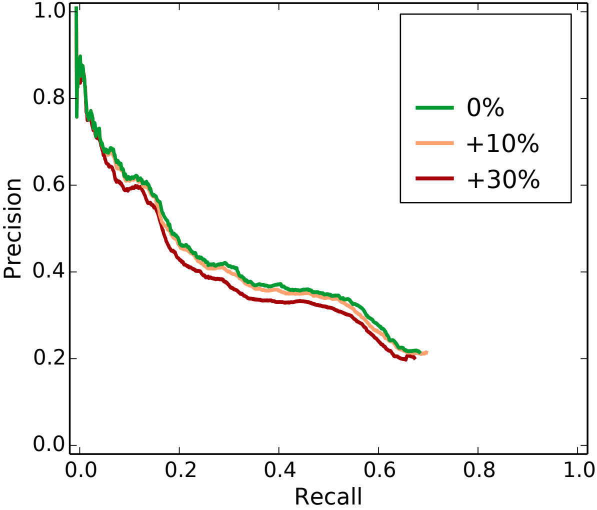

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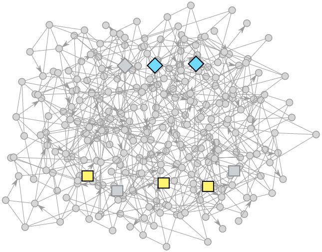

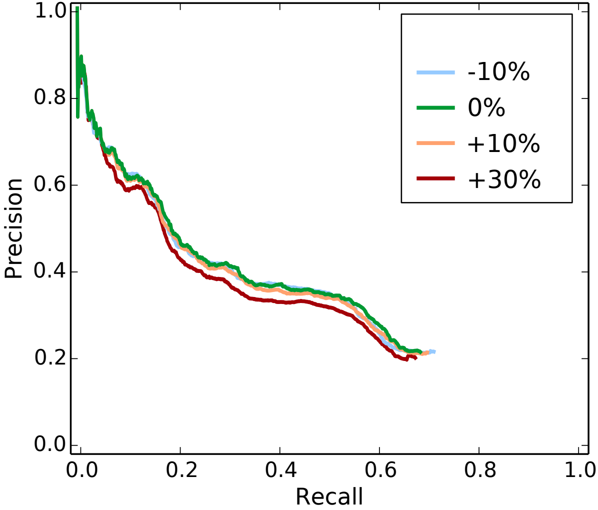

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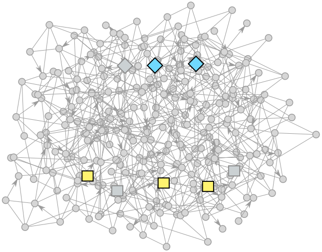
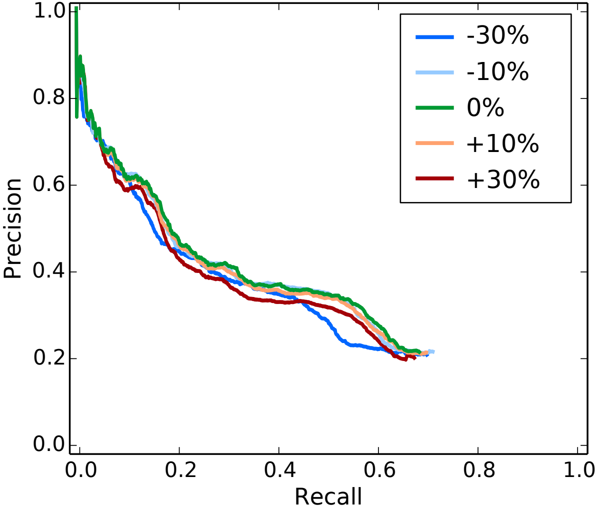
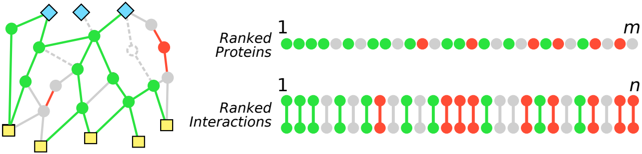
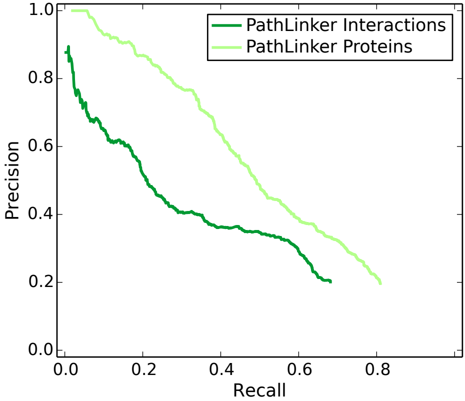




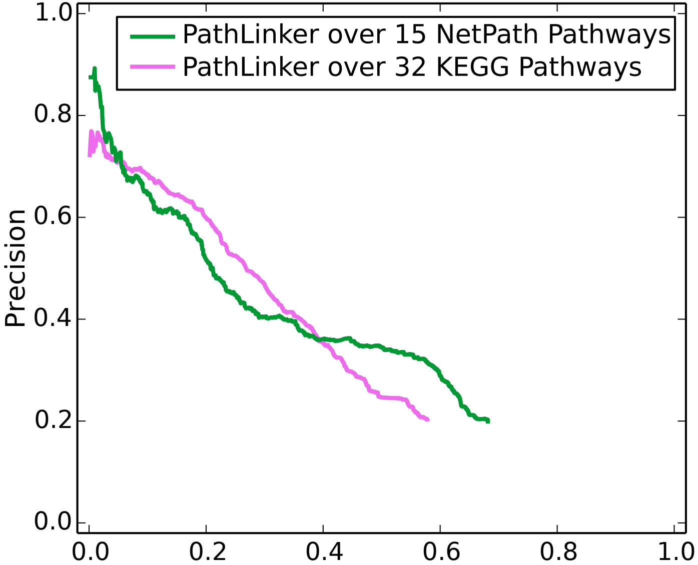
# Compare to Reconstructing Proteins



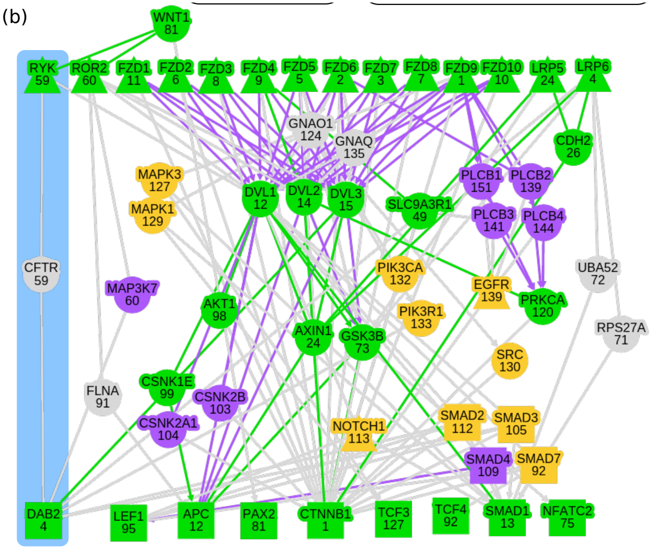


# Reconstruct KEGG Pathways

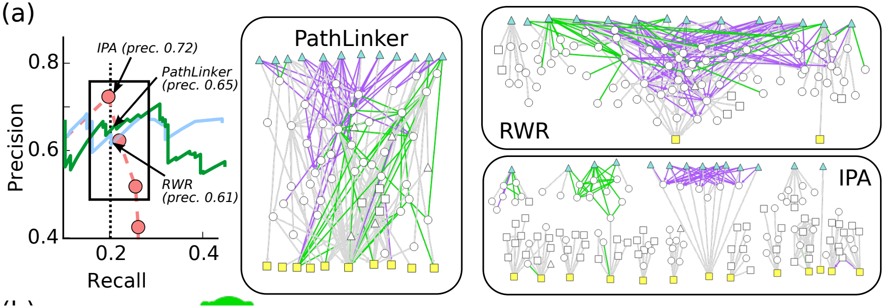
▸ List of KEGG Pathways

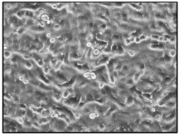


# Wnt Signaling Pathway: Top 200 PathLinker Paths

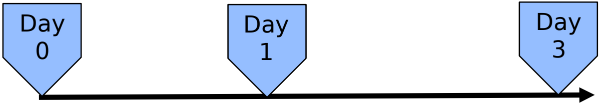


# Comparing Wnt Reconstructions





HEK293 Cells



Day 0

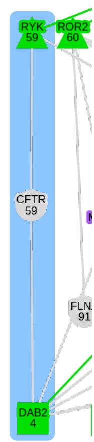
Plate cells and siRNA silence gene of interest

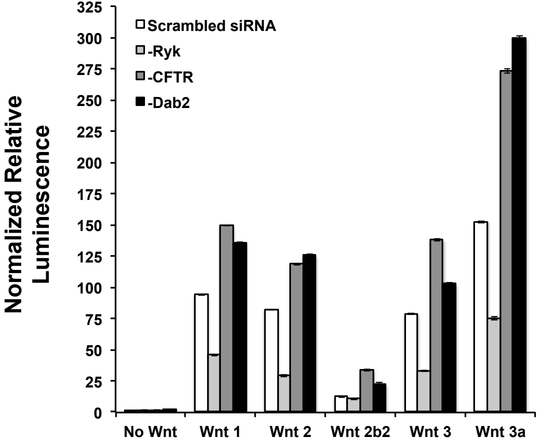
Day 1

Transfect secreted Wnt3a (sWnt3a) stimulus **and Luciferase reporter**

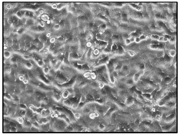
Day 3

Measure **luminescence**





▸ Luciferase Reporter Efficacy ▸ siRNA Silencing Efficacy ▸ CoIP



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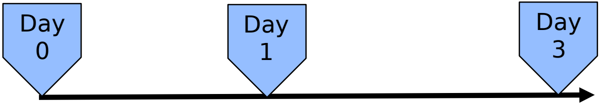

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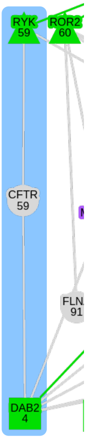

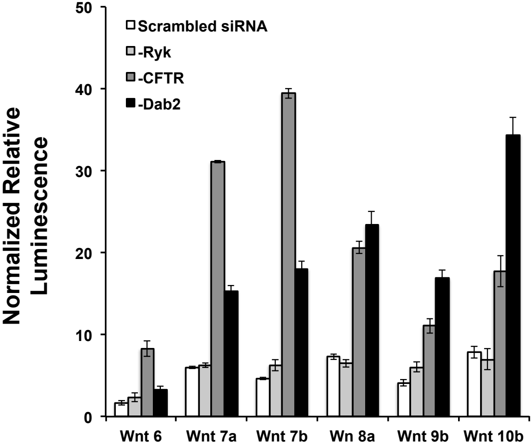

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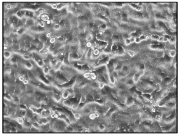


HEK293 Cells

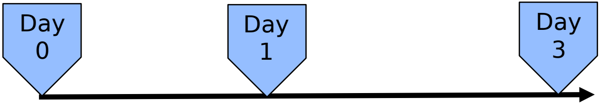
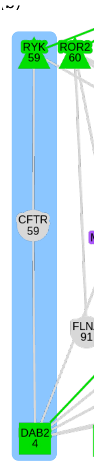
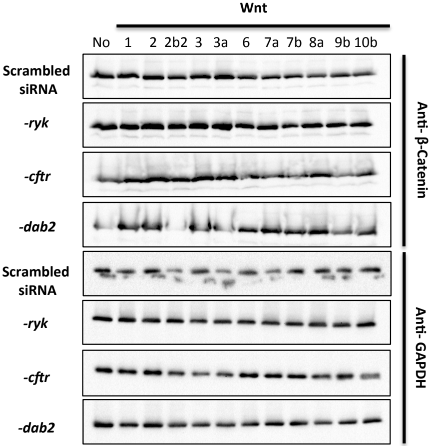


Plate cells and siRNA silence gene of interest

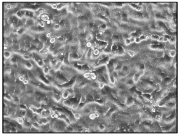
Transfect secreted Wnt3a (sWnt3a) stimulus

Measure **cellular B-catenin levels**

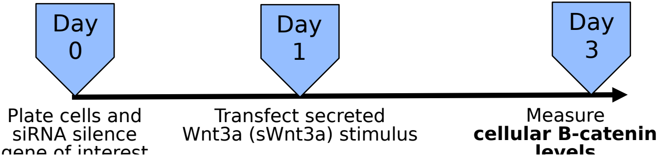




▸ Luciferase Reporter Efficacy ▸ siRNA Silencing Efficacy ▸ CoIP



HEK293 Cells



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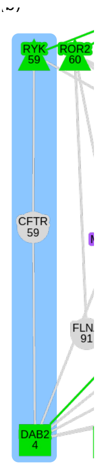
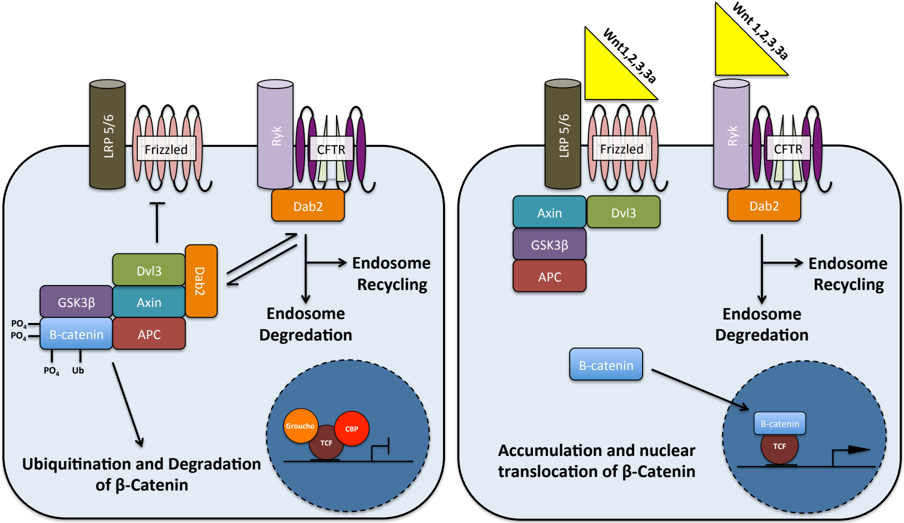


| | **Control** | | **-Ryk** | | **-CFTR** | | **-Dab2** | |
|---|---|---|---|---|---|---|---|---|
| **Wnt** | **NRL** | **QNβ** | **NRL** | **QNβ** | **NRL** | **QNβ** | **NRL** | **QNβ** |
| No Wnt | -- | -- | -- | -- | -- | -- | -- | -- |
| Wnt 1 | VS | ++ | S | - | VS | + | VS | ++ |
| Wnt 2 | VS | + | S | ++ | VS | ++ | VS | ++ |
| Wnt 2b2 | W | - | W | + | S | ++ | W | - |
| Wnt 3 | VS | ++ | S | ++ | VS | ++ | VS | ++ |
| Wnt 3a | VS | ++ | VS | ++ | VS | ++ | VS | ++ |
| Wnt 6 | W | ++ | W | + | W | + | W | ++ |
| Wnt 7a | W | - | W | + | S | - | W | ++ |
| Wnt 7b | W | ++ | W | - | S | - | W | ++ |
| Wn 8a | W | - | W | - | W | ++ | W | ++ |
| Wnt 9b | W | - | W | - | W | - | W | ++ |
| Wnt 10b | W | - | W | - | W | ++ | S | ++ |

▸ *Luciferase Reporter Efficacy* ▸ *siRNA Silencing Efficacy* ▸ *CoIP*

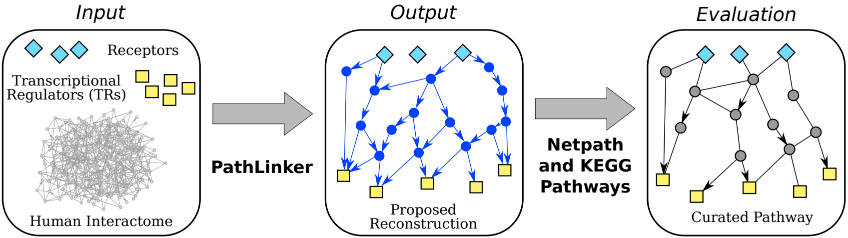
# New Model: Dvl is an Amplifier of Wnt Signaling



- Wnt Signaling

\+ Wnt 1,2,3,3a Signaling

# PathLinker Summary



*Input*

Receptors

Transcriptional Regulators (TRs)

Human Interactome

**PathLinker**

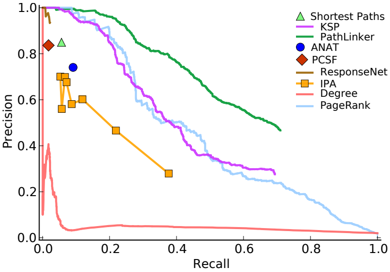
*Output*

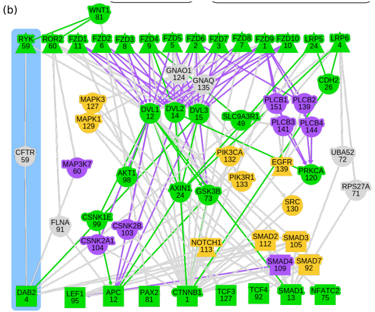
Proposed Reconstruction

**Netpath and KEGG Pathways**

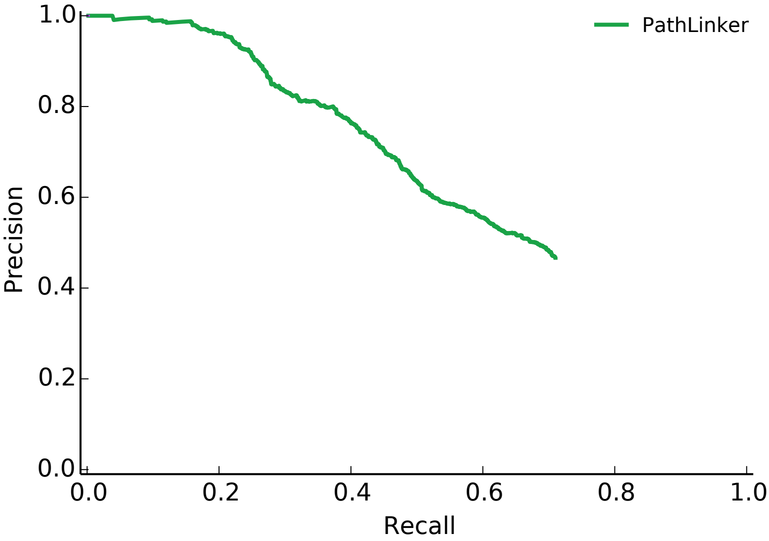
*Evaluation*

Curated Pathway

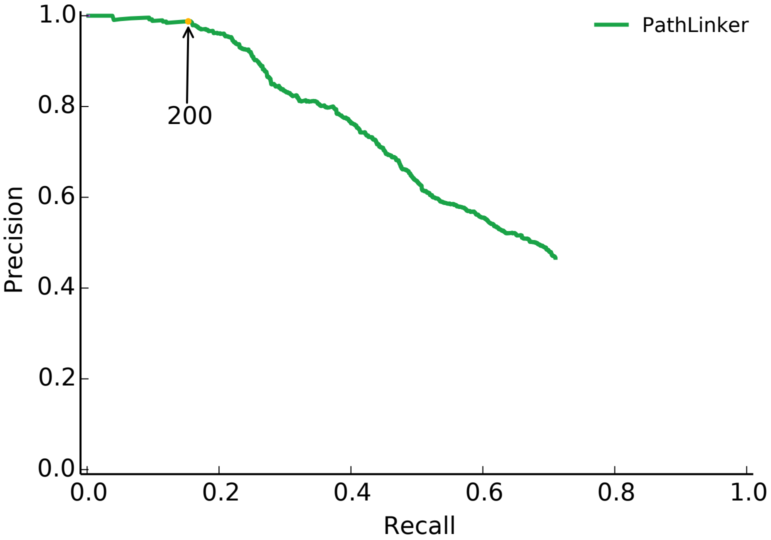




# How Many Paths Does PathLinker Need to Compute?



# How Many Paths Does PathLinker Need to Compute?



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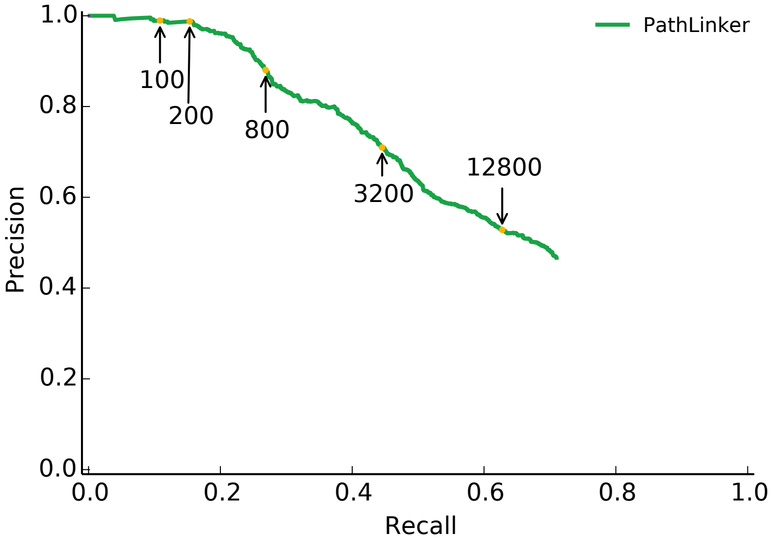
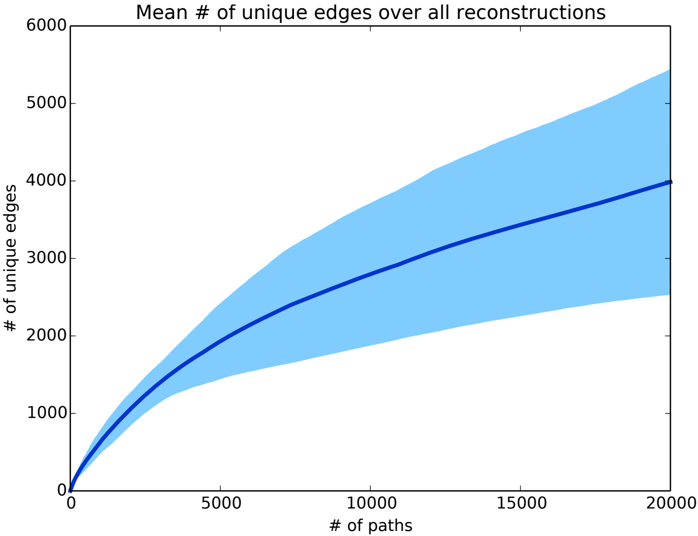


We compute 20,000 paths to achieve a recall of 0.7.

▸ Performance

# How Many Paths Does PathLinker Need to Compute?



We compute 20,000 paths to achieve a recall of 0.7.

▸ Performance

# NetPath Pathways

1. The pathway contains at least one receptor.
2. The pathway contains at least one TR, and
3. The minimum cut between the receptors and TRs was at least three in the NetPath pathway.

| **Pathway** | #Nodes | #Edges | Min Cut | # Receptors | # TRs |
|---|---|---|---|---|---|
| BDNF | 72 | 139 | 4 | 5 | 4 |
| EGFR1 | 231 | 1456 | 30 | 6 | 33 |
| IL1 | 43 | 178 | 7 | 3 | 5 |
| IL2 | 67 | 242 | 16 | 3 | 12 |
| IL3 | 70 | 176 | 5 | 2 | 9 |
| IL6 | 53 | 162 | 6 | 4 | 14 |
| IL7 | 18 | 52 | 5 | 2 | 3 |
| Kit Receptor | 76 | 207 | 5 | 6 | 8 |
| Leptin | 55 | 135 | 8 | 3 | 15 |
| Prolactin | 68 | 199 | 10 | 4 | 9 |
| RANKL | 57 | 142 | 4 | 2 | 12 |
| TCR | 154 | 504 | 8 | 4 | 21 |
| TGF$\beta$ Receptor | 209 | 863 | 32 | 5 | 78 |
| TNF$\alpha$ | 239 | 913 | 15 | 4 | 44 |
| Wnt | 106 | 428 | 7 | 14 | 14 |

◂ Inputs for Pathway Reconstruction

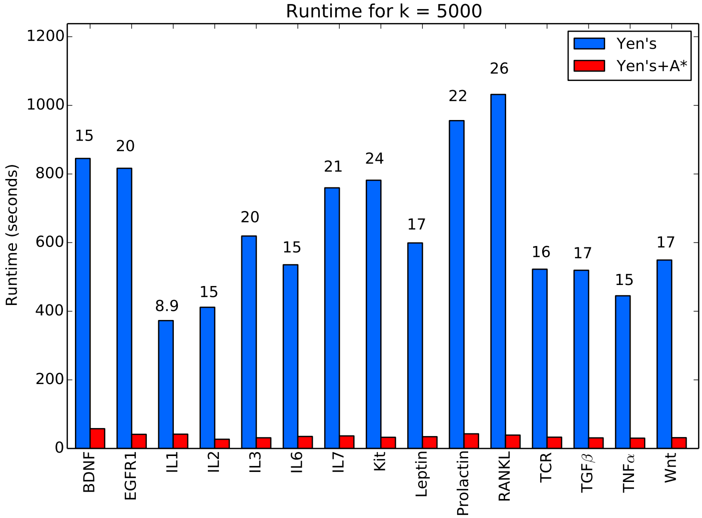
# KEGG Pathways

◂ KEGG Results

1. The Pathway is related to signaling.
2. The pathway contains at least one receptor.
3. The pathway contains at least one TR, and
4. The minimum cut between the receptors and TRs was $\geq 3$ in the KEGG pathway.

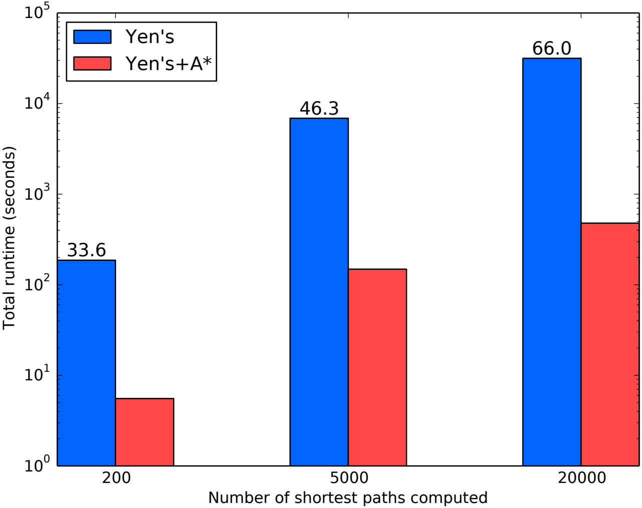
| Name | KEGG ID | Name | KEGG ID |
|---|---|---|---|
| Adherens junction | `hsa04520` | Adipocytokine signaling pathway | `hsa04920` |
| Apoptosis | `hsa04210` | Axon guidance | `hsa04360` |
| Chemokine signaling pathway | `hsa04062` | Circadian entrainment | `hsa04713` |
| Dopaminergic synapse | `hsa04728` | Endocytosis | `hsa04144` |
| ErbB signaling pathway | `hsa04012` | Focal adhesion | `hsa04510` |
| FoxO signaling pathway | `hsa04068` | GnRH signaling pathway | `hsa04912` |
| HIF-1 signaling pathway | `hsa04066` | Hippo signaling pathway | `hsa04390` |
| Insulin signaling pathway | `hsa04910` | Jak-STAT signaling pathway | `hsa04630` |
| Prolactin signaling pathway | `hsa04917` | MAPK signaling pathway | `hsa04010` |
| Melanogenesis | `hsa04916` | Natural killer cell mediated cytotoxicity | `hsa04650` |
| Neurotrophin signaling pathway | `hsa04722` | NF-kappa B signaling pathway | `hsa04064` |
| Notch signaling pathway | `hsa04330` | Osteoclast differentiation | `hsa04380` |
| TGF-beta signaling pathway | `hsa04350` | Thyroid hormone signaling pathway | `hsa04919` |
| Tight junction | `hsa04530` | Toll-like receptor signaling pathway | `hsa04620` |
| VEGF signaling pathway | `hsa04370` | Wnt signaling pathway | `hsa04310` |
| Leukocyte transendothelial migration | `hsa04670` | Signaling pathways regulating pluripotency of stem cells | `hsa04550` |

# PathLinker Performance



▸ How Many Paths?

# PathLinker Performance



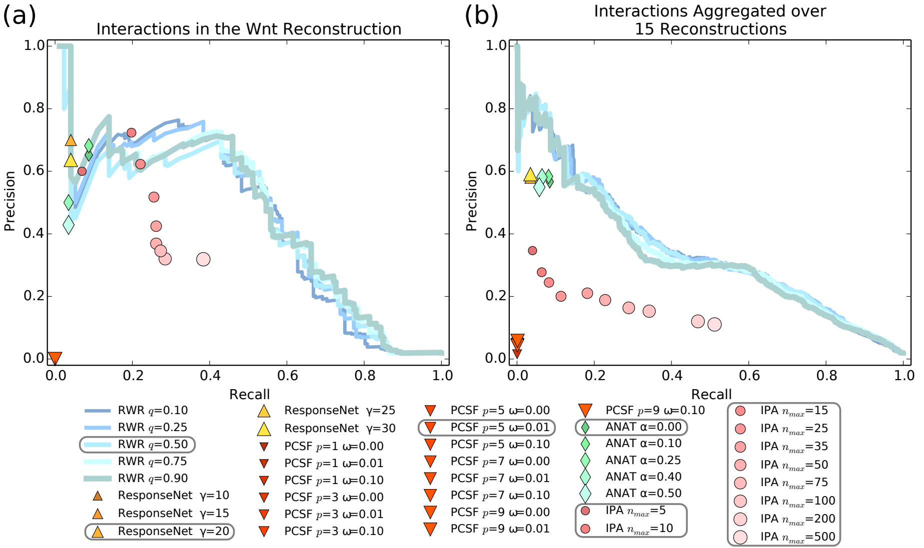
▸ How Many Paths?

# Algorithm Internal Parameters

| Algorithm | Parameter | Meaning |
|---|---|---|
| PathLinker | $k$ | Number of shortest paths |
| RWR | $q$ | Teleportation probability |
| ANAT | $\alpha$ | Tradeoff between global (Steiner tree) and local (shortest path) solution |
| PCSF | $\omega$ | Penalty for adding a new tree |
|  | $p$ | Prize for each node |
| ResponseNet | $\gamma$ | Number of interactions that carry flow |
| IPA | $n_{max}$ | Maximum sub-network size |

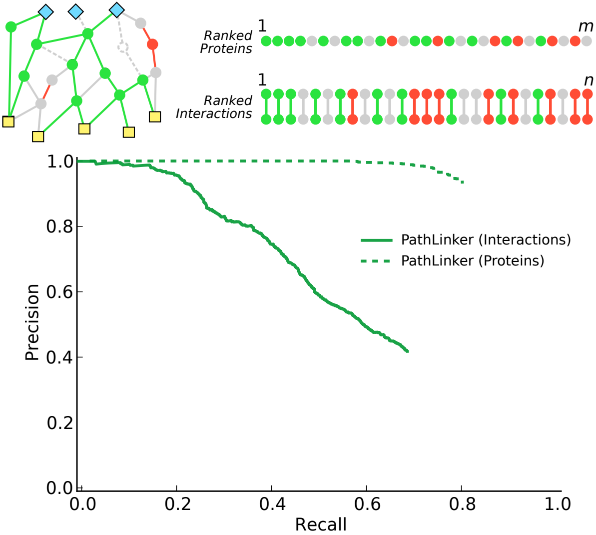
◂ PathLinker Summary

# Algorithm Internal Parameters



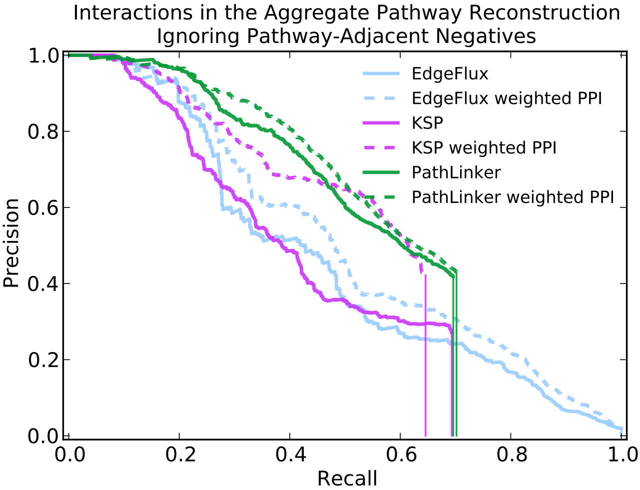
◂ PathLinker Summary

# Recovering Proteins in a Pathway



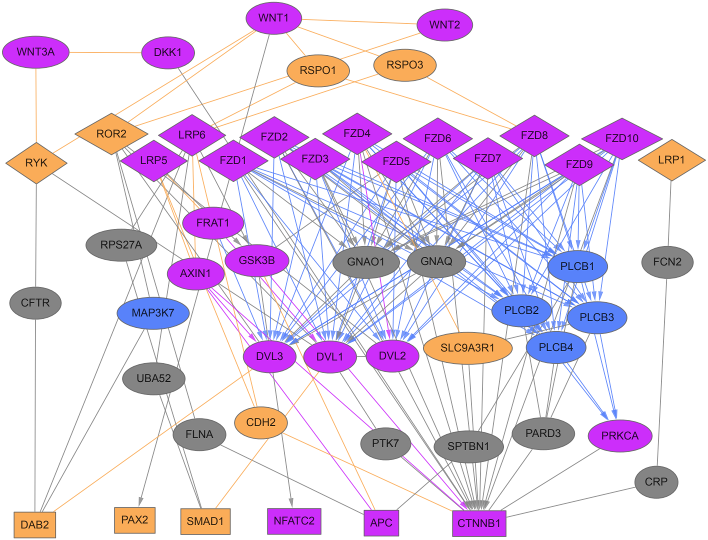
▸ PathLinker Summary

# PathLinker on a Weighted PPI

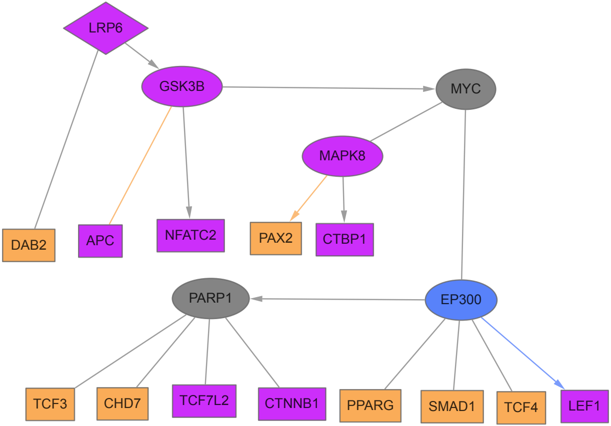


▸ PathLinker Summary

# PathLinker Network

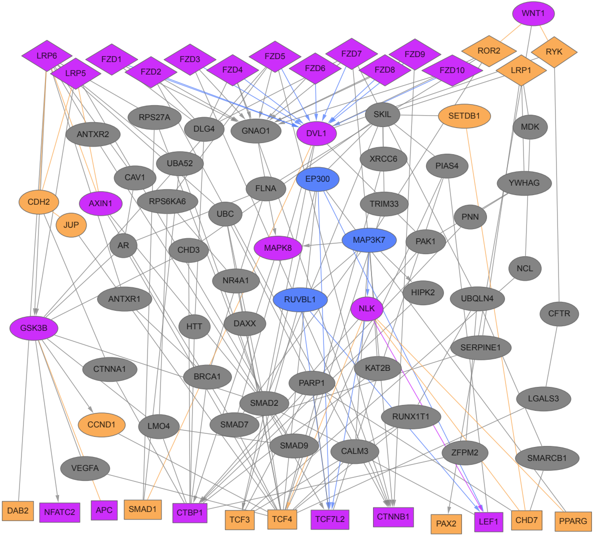


# PCSF Network



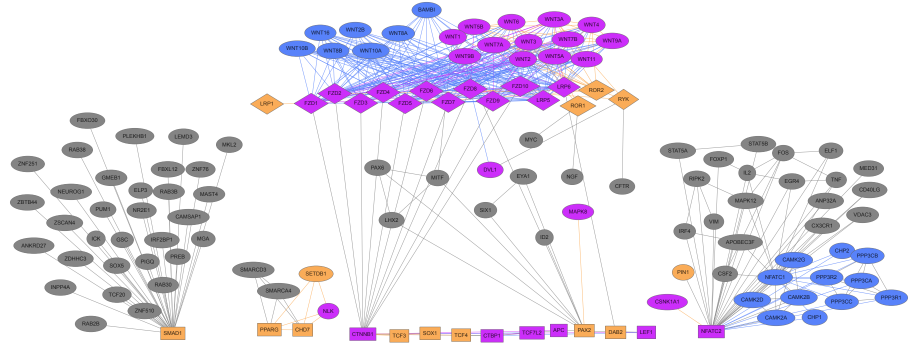
▸ PathLinker Network

# ANAT Network



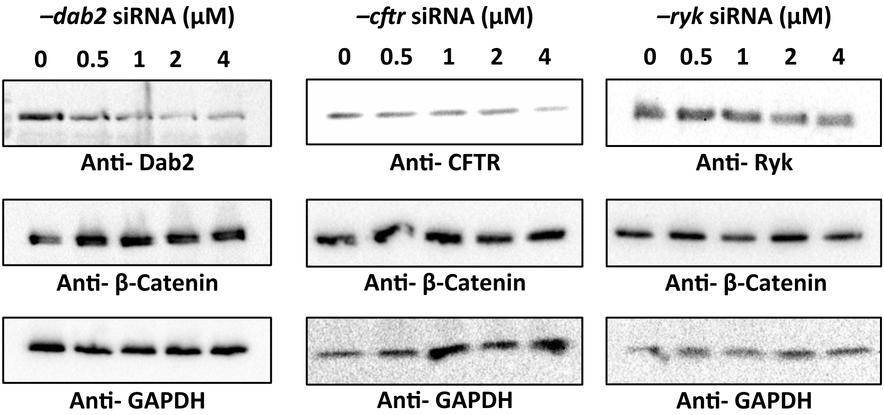
▸ PathLinker Network

# IPA Network



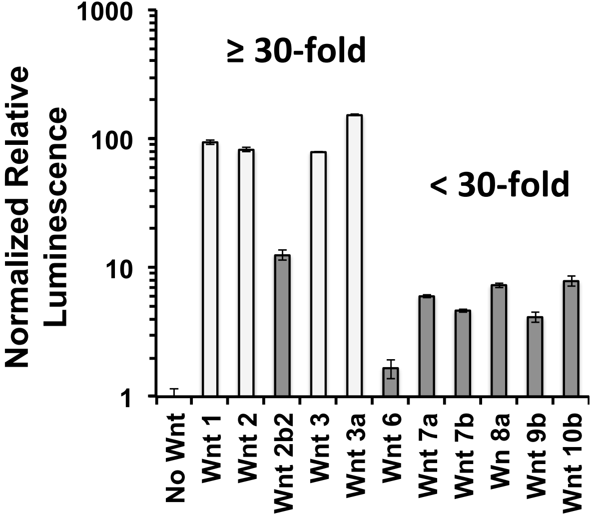
▸ PathLinker Network

# Luciferase Reporter Efficacy



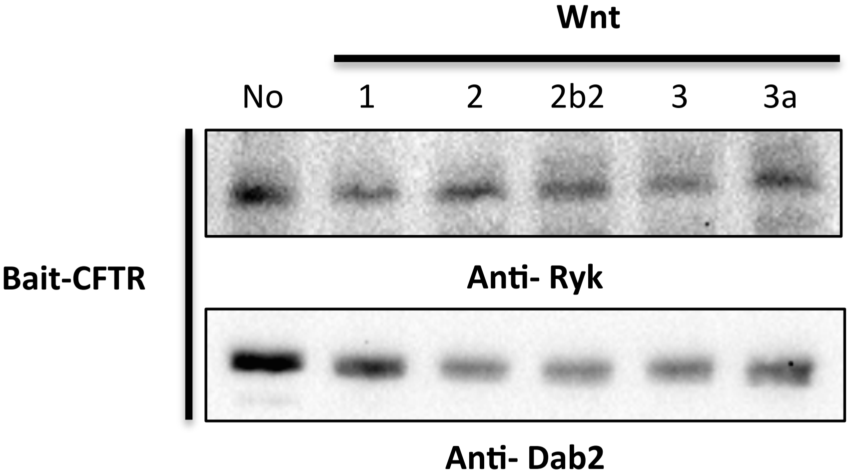
▸ siRNA Experiments

# siRNA Silencing Efficacy



▸ sWnt3a Experiments

# Co-Immunoprecipitation Experiments



▸ sWnt3a Experiments